



**Faculty of Resource Science and Technology**

**Bioactivities, Phytochemicals and Pharmacophore Analysis of Nipa Palm  
(*Nypa fruticans* Wurmb) Sugar Extracts**

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Bioactivities, Phytochemicals and Pharmacophore Analysis of Nipa Palm  
(*Nypa fruticans* Wurmb) Sugar Extracts

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## **DECLARATION**

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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## ABSTRACT

Nipa palm (*Nypa fruticans* Wurmb) sugar is used as a sweetener for local people especially in Sarawak. It was processed by using nipa palm sap as a raw material as it was cooked for several hours until it became brown syrup. Nipa palm sugar or widely known as gula apong. Nipa palm (*N. fruticans* Wurmb) sugar was extracted by using different solvents which were methanol (MeOH), ethanol (EtOH), water (H<sub>2</sub>O), ethyl acetate (EtOAc) and hexane (Hex). All of the extracts were used to determine the moisture content, ash content, pH of the nipa palm sugar. Phytochemical screening was conducted in order to know the presence of the active compounds in the extracts. MeOH, EtOH and H<sub>2</sub>O extracts were tested positive for the presence of saponins, tannins, terpenoids, flavonoids, phenolic compounds, reducing sugar and non-reducing sugar. The antioxidant activity of extracts was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and the results showed that nipa palm sugar extracts possessed antioxidant properties which were EtOH (IC<sub>50</sub> = 200.6 µg/mL), MeOH (IC<sub>50</sub> = 208.3 µg/mL), H<sub>2</sub>O (IC<sub>50</sub> = 569.8 µg/mL), EtOAc (IC<sub>50</sub> = 1336.0 µg/mL) and Hex (IC<sub>50</sub> = 4917.0 µg/mL). Alpha-amylase inhibition assay was performed by comparing with the acarbose and the results for the extracts were EtOH (IC<sub>50</sub> = 109.40 µg/mL), MeOH (IC<sub>50</sub> = 124.20 µg/mL) and H<sub>2</sub>O (IC<sub>50</sub> = 545.10 µg/mL). The antioxidant activity and alpha-amylase inhibition assay resulted that EtOH extract showed high inhibition for both assay. All of the extracts showed the low toxicity level against the brine shrimp (*Artemia salina*). The LigandScout 4.3 software based on Computer-Aided Drug Design (CADD) was used to identify the value of the nipa palm sugar in the pharmaceutical industry. Based on the ligand-based pharmacophore analysis, arginine showed the highest fit-value compared to other chemical constituents in nipa palm sugar, which is 89.42, with the interactions of 4 HBA, 4 HBD and 1 PI. Structure-based

pharmacophore modelling was generated based on a 3D crystal structure of human pancreatic alpha-amylase which had been retrieved from Protein Data Bank (PDB code: 1B2Y) and resulted that arginine compound of nipa palm sugar had the highest score for alignment score compared to other test sets which is 106.41 and it possessed two types of interactions which were HBA (ARG195A and HOH930A) and HBD (ASP300A, GLY233A, HIS299A and HOH610A). Thus, it was concluded that nipa palm sugar compounds showed that its compounds possess a great potential for inhibiting alpha-amylase.

**Keywords:** *Nypa fruticans* Wurmb, DPPH, alpha-amylase, CADD, ligand-based pharmacophore analysis

***Bioaktiviti, Fitokimia dan Analisis Farmakofor terhadap Ekstrak Gula Pokok Nipah  
(Nypa fruticans Wurmb)***

***ABSTRAK***

*Gula nipah (Nypa fruticans Wurmb) digunakan sebagai pemanis oleh orang tempatan terutamanya di Sarawak. Gula nipah diproses dengan menggunakan air nira sebagai bahan mentah, ia dimasak selama beberapa jam sehingga menjadi warna coklat dalam keadaan sirap. Gula nipah juga dikenali sebagai gula apung. Gula nipah (Nypa fruticans Wurmb) diekstrak dengan menggunakan pelbagai pelarut iaitu metanol (MeOH), etanol (EtOH), air suling (H<sub>2</sub>O), etil asetat (EtOAc) dan juga heksana (Hex). Kesemua ekstrak digunakan untuk menentukan tahap kelembapan, kadar kandungan abu dan pH gula nipah. Saringan fitokimia telah dijalankan bagi mengetahui kewujudan kompaun aktif yang berada di dalam ekstrak. MeOH, EtOH dan H<sub>2</sub>O ekstrak didapati positif untuk kewujudan kompaun saponin, tanin, terpenoid, flavonoid, kompaun fenolik, kekurangan gula dan juga tidak mengurangkan gula. Tindakan antioksidan juga telah dinilai menggunakan 2,2-diphenyl-1-picrylhydrazyl (DPPH) untuk mengikis aktiviti radikal bebas dan keputusan menunjukkan bahawa ekstrak gula nipah mempunyai antioksidan yang mana ekstrak EtOH (IC<sub>50</sub> = 200.6 µg/mL), MeOH (IC<sub>50</sub> = 208.3 µg/mL), H<sub>2</sub>O (IC<sub>50</sub> = 569.8 µg/mL), EtOAc (IC<sub>50</sub> = 1336.0 µg/mL) dan Hex (IC<sub>50</sub> = 4917.0 µg/mL). Aktiviti perencatan enzim amilase telah dilaksanakan dengan membandingkan dengan acarbose dan keputusan untuk ekstrak adalah EtOH (IC<sub>50</sub> = 109.40 µg/mL), MeOH (IC<sub>50</sub> = 124.20 µg/mL) and H<sub>2</sub>O (IC<sub>50</sub> = 545.10 µg/mL). Aktiviti antioksidan dan perencatan enzim amilase menunjukkan bahawa ekstrak EtOH memberi perencatan yang tinggi untuk kedua-dua aktiviti tersebut. Kesemua ekstrak menunjukkan kadar toksik yang rendah terhadap udang air garam (Artemia salina). Perisian LigandScout 4.3 berdasarkan CADD telah digunakan untuk mengenalpasti nilai gula nipah dalam bidang*

*farmasi. Berdasarkan analisis ligan farmakofor, arginin menunjukkan nilai yang paling tertinggi berbanding dengan komponen kimia yang lain di gula nipah, yaitu pada nilai 89.42, dengan interaksi 4 HBA, 4 HBD dan 1 PI. Pemodelan farmakofor berdasarkan struktur telah dihasilkan berdasarkan struktur kristal 3D alpha-amilase pancreas manusia yang diambil daripada Bank Data Protein (kod PDB: 1B2Y) dan terhasil bahawa sebatian arginin gula apong mempunyai skor tertinggi untuk skor penjajaran berbanding dengan set ujian yang lain iaitu dengan skor 106.41 dan ia mempunyai dua jenis interaksi iaitu HBA (ARG195A dan HOH930A) dan HBD (ASP300A, GLY233A, HIS299A dan HOH610A). Dengan demikian, ia disimpulkan bahawa sebatian dalam gula apong menunjukkan bahawa sebatianannya memiliki potensi yang besar untuk merencatkan alpha-amilase.*

**Kata kunci:** *Nypa fruticans Wurmb, DPPH, enzim amilase, CADD, analisis ligan farmakofor*



## TABLE OF CONTENTS

	Page
<b>DECLARATION</b>	i
<b>ACKNOWLEDGEMENT</b>	ii
<b>ABSTRACT</b>	iii
<b><i>ABSTRAK</i></b>	v
<b>TABLE OF CONTENTS</b>	vii
<b>LIST OF TABLES</b>	xi
<b>LIST OF FIGURES</b>	xiii
<b>LIST OF ABBREVIATIONS</b>	xvi
<b>CHAPTER 1: INTRODUCTION</b>	1
1.1 Study Background	1
1.2 Problem Statement	3
1.3 Objectives	4
<b>CHAPTER 2: LITERATURE REVIEW</b>	5
2.1 <i>Nypa fruticans</i> Wurmb	5
2.1.1 Plant Profile	5
2.1.2 Vernacular Names of <i>Nypa fruticans</i> Wurmb	6
2.1.3 The Uses of <i>Nypa fruticans</i> Wurmb	7
2.2 Phytochemical Analysis of <i>N. fruticans</i> Wurmb	7

2.3	Secondary Metabolites	8
2.3.1	Phenolic Compounds	8
2.3.2	Flavonoids	10
2.3.3	Tannins	12
2.3.4	Glycosides	13
2.3.5	Saponins	13
2.4	Previous Studies on <i>Nypa fruticans</i> Wurmb	14
2.4.1	Antioxidant Studies	14
2.4.2	Compounds in Nipa Palm Sugar of <i>N. fruticans</i> Wurmb	15
2.4.3	Diabetes Mellitus	17
2.5	Computer-Aided Drug Design	18
2.5.1	Pharmacophore Modelling	20
2.5.2	Pharmacophore Model Based Virtual Screening	21
2.5.3	Ligand-Based Drug Design (LBDD)	23
2.5.4	Structure-Based Drug Design (SBDD)	24
	<b>CHAPTER 3: METHODOLOGY</b>	26
3.1	General Procedure	26
3.2	Preparation of Sample	26
3.2.1	Sample Collection	26
3.2.2	Sample Extraction	27

3.3	Determination of Moisture Content, Ash Content and pH	28
3.3.1	Determination of Moisture Content	28
3.3.2	Determination of Ash Content	29
3.3.3	Determination of pH	30
3.4	Phytochemical Qualitative Analysis of Nipa Palm Sugar Extracts	30
3.4.1	Test for Saponins	30
3.4.2	Test for Tannins	31
3.4.3	Test for Phlobatannins	31
3.4.4	Test for Terpenoids	31
3.4.5	Test for Flavonoids	32
3.4.6	Test for Phenolic Compounds	32
3.4.7	Test for Reducing Sugar	32
3.4.8	Test for Non-Reducing Sugar	33
3.5	Biological Assay	33
3.5.1	DPPH Radical Scavenging Assay	33
3.5.2	Alpha-Amylase Inhibitory Activity	34
3.5.3	Brine Shrimp Lethality Assay	36
3.6	Pharmacophore Modelling	37
3.6.1	Ligand-Based Pharmacophore Model	38
3.6.2	Structure-Based Pharmacophore Analysis	45

<b>CHAPTER 4: RESULTS AND DISCUSSION</b>	<b>50</b>
4.1 Sample Extraction	50
4.2 Determination of Moisture Content, Ash Content and pH	50
4.3 The Phytochemical Qualitative Analysis of Nipa Palm Sugar Extracts	52
4.4 Biological Assays	55
4.4.1 DPPH Radical Scavenging Assay	55
4.4.2 Alpha –Amylase Inhibitory Activity	59
4.4.3 Brine Shrimp Lethality Assay	63
4.5 Pharmacophore Modelling	66
4.5.1 Ligand-Based Pharmacophore Analysis	66
4.5.2 Structure-Based Pharmacophore Analysis	79
<b>CHAPTER 5: CONCLUSION AND RECOMMENDATIONS</b>	<b>91</b>
5.1 Conclusion	91
5.2 Recommendations	93
<b>REFERENCES</b>	<b>94</b>
<b>APPENDICES</b>	<b>112</b>

## LIST OF TABLES

	<b>Page</b>
Table 2.1 The taxonomy of <i>N. fruticans</i> Wurmb (CABI, 2006)	6
Table 2.2 The vernacular names of <i>Nypa fruticans</i> Wurmb (Baja-Lapis et al., 2004)	6
Table 2.3 The phytochemical screening of <i>N. fruticans</i> Wurmb leaves (Basyuni et al., 2019)	8
Table 2.4 The reported compound in nipa palm sugar of <i>N. fruticans</i> Wurmb	16
Table 3.1 The pharmacophore features which include the colour of the features	42
Table 4.1 The moisture content, ash content and pH of the nipa palm sugar extracts	50
Table 4.2 The moisture content, ash content and pH of nipa palm ( <i>N. fruticans</i> Wurmb) sugar extracts	51
Table 4.3 Results for phytochemical screening of <i>Nypa fruticans</i> Wurmb sugar extracts	52
Table 4.4 The presence of reducing sugar and non-reducing sugar in nipa palm sugar (Radam et al., 2016; Phetrit et al., 2020)	55
Table 4.5 The percentage inhibition and IC <sub>50</sub> of <i>N. fruticans</i> Wurmb sugar extracts	56
Table 4.6 The percentage inhibition and IC <sub>50</sub> of <i>N. fruticans</i> Wurmb sugar extracts	60
Table 4.7 The results of brine shrimp lethality assay	64
Table 4.8 The list of generated models for ligand-based screening	67
Table 4.9 The features that describe the differences type of interactions	69

Table 4.10	The summary results for ligand-based pharmacophore analysis	78
Table 4.11	Summary interactions of the training sets and test sets in active site	88
Table 4.12	Evaluation of the test sets based on Lipinski's rule	90

## LIST OF FIGURES

	<b>Page</b>
Figure 2.1 <i>Nypa fruticans</i> Wurmb's tree	5
Figure 2.2 The chemical structure of phenolic compounds which can be found in unripe endosperm of <i>Nypa fruticans</i> Wurmb (Prasad et al., 2013)	10
Figure 2.3 The chemical structure of the subgroups of flavonoids (Panche et al., 2016)	11
Figure 2.4 The chemical structure of diosgenin and hecogenin (Doughari, 2012)	14
Figure 2.5 The application of CADD for the various stages of drug development (Kore et al., 2012)	19
Figure 2.6 The workflow of pharmacophore-based virtual screening (Langer & Welber, 2004)	22
Figure 3.1 Nipa palm sugar	27
Figure 3.2 The chemical structure of the training sets	39
Figure 3.3 The chemical structure of test sets (Demann, 1999; Ho et al., 2008)	41
Figure 3.4 The flowchart of ligand-based pharmacophore analysis	45
Figure 3.5 A 3D crystal structure of human pancreatic $\alpha$ -amylase which generated by LigandScout 4.3 software	46
Figure 3.6 The flowchart of structure-based pharmacophore analysis	49
Figure 4.1 The percentage of inhibition against the concentration of extracts	58
Figure 4.2 The percentage of inhibition of $\alpha$ -amylase against the log concentration of extracts	61
Figure 4.3 The percentage of mortality of brine shrimp against the log concentration of nipa palm extracts	65

Figure 4.4	The pharmacophore model which is being generated by the selected training sets	68
Figure 4.5	The interaction of voglibose toward the generated pharmacophore model	69
Figure 4.6	The interaction of acarbose toward the generated pharmacophore model	70
Figure 4.7	The interaction of miglitol toward the generated pharmacophore model	71
Figure 4.8	The interaction of arginine toward the generated pharmacophore model	71
Figure 4.9	The interaction of glutamine toward the generated pharmacophore model	72
Figure 4.10	The interaction of asparagine toward the generated pharmacophore model	72
Figure 4.11	The interaction of 4-hydroxyhexane-2,3,5-trione toward the generated pharmacophore model	73
Figure 4.12	The interaction of vanillic acid and the generated pharmacophore model	74
Figure 4.13	The interaction of 2-hydroxycinnamic acid toward the generated pharmacophore model	74
Figure 4.14	The interaction of 4-hydroxy-2,5-dimethyl-3(2H)-furanone toward the generated pharmacophore model	75
Figure 4.15	The interaction of diacetyl toward the generated pharmacophore model	76



Figure 4.16	The interaction of formic acid toward the generated pharmacophore model	76
Figure 4.17	The active site of crystal structure of the human pancreatic alpha-amylase	79
Figure 4.18	Structure-based pharmacophore model generated by LigandScout 4.3	80
Figure 4.19	The structure-based pharmacophore analysis for acarbose	81
Figure 4.20	The structure-based pharmacophore analysis for voglibose	82
Figure 4.21	The structure-based pharmacophore analysis of miglitol	83
Figure 4.22	The structure-based pharmacophore analysis of arginine	83
Figure 4.23	The structure-based pharmacophore analysis of glutamine	84
Figure 4.24	The structure-based pharmacophore analysis of asparagine	84
Figure 4.25	The structure-based pharmacophore analysis of 4-hydroxyhexane-2,3,3-trione	85
Figure 4.26	The structure-based pharmacophore analysis of 2-hydroxycinnamic	85
Figure 4.27	The structure-based pharmacophore analysis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone	86
Figure 4.28	The structure-based pharmacophore analysis of diacetyl	87
Figure 4.29	The structure-based pharmacophore analysis of formic acid	87
Figure 4.30	The structure-based pharmacophore analysis of vanillic acid	87

## LIST OF ABBREVIATIONS

AR	Aromatic
CADD	Computer Aided Drug Design
CHCl <sub>3</sub>	Chloroform
DMSO	Dimethyl sulfoxide
DNS	3,5-dinitrosalicylic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EtOAc	Ethyl acetate
EtOH	Ethanol
FeCl <sub>3</sub>	Iron (III) chloride
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
HBA	Hydrogen Bond Acceptor
HBD	Hydrogen Bond Donor
HEX	Hexane
HY	Hydrophobic
LC <sub>50</sub>	Lethal Concentration 50
MeOH	Methanol
NH <sub>3</sub>	Ammonia
nm	Nanometre
PI	Positive Ionisable
SBDD	Structure Based Drug Design
UV-Vis	Ultraviolet–visible
μL	Microliter

## CHAPTER 1

### INTRODUCTION

#### 1.1 Study Background

One of the most common and widely distributed in the mangrove forests of Southeast Asia is Nipa Palm (*Nypa fruticans* Wurmb). *N. fruticans* Wurmb found in the upstream estuarine zone which forms extensive stands along brackish to tidal freshwater creeks and rivers (Tamunaidu & Saka, 2011). It grows really fast especially in freshwater. *N. fruticans* Wurmb belong to the family of Arecaceae (Prasad et al., 2013). According to Duke (2006), the generic name of *Nypa* is derivative of “Nipah” which is the native name that was used in the Philippines and the Moluccas. “Fruticans” is a Latin word for shrubby which refers to its stem less appearance. *N. fruticans* Wurmb habitat is unique as it does not compete with food crops and its growth is sustainable. In addition, it also lacks an upright stem, which is one of the characteristic features for the palms.

The nipa palm sap is a good source of sugar and it is used for making sweets (nipa palm sugar), beverages, vinegar and alcohol (Joshi et al., 2006). The nipa palm sap was harvested from inflorescence (flower cluster) by making a cut on the inflorescence. The nipa palm sap was flown into a bamboo container which had been used by villagers in order to collect nipa palm sap. The nipa palm sap was boiled until the colour of the nipa palm sugar turned to brown syrup (Apriyantono et al., 2002). In the Southeast and South Asian regions such as Malaysia, Indonesia, Thailand, Philippines and India, nipa palm sugar has been used as a traditional and an alternative sweetener as the nipa palm (*Nypa fruticans* Wurmb) are found in abundance (Saputro et al., 2009).

In this study, the nipa palm (*N. fruticans* Wurmb) sugar extracts was analysed to determine the moisture content, ash content and pH. Hence, the phytochemical analysis was conducted in order to detect the presence of the saponins, tannins, phlobatannins, phenolic, terpenoids, reducing sugar and non-reducing sugar compounds in nipa palm sugar. Hence, the biological assay was conducted such as DPPH radical scavenging activity, alpha-amylase inhibition activity and brine shrimp lethality assay against the nipa palm sugar extracts.

According to Kanatt et al. (2007), the antioxidants from plant sources have been an increasing concern to the consumers since synthetic antioxidants such as butylated hydroxytoluene (BHT) have restricted usage in foods, because of the carcinogenic activity. Hence, the epidemiological studies have shown that frequent consumption of fruits and vegetables high in natural antioxidants can lower the incidence of certain types of cancer, cardiovascular diseases, and diabetes (Zambonin et al., 2012). These beneficial effects are related to bioactive compounds like phenolic acids, flavonoids, anthocyanin and carotenoids possessing antioxidant activity (Gordon et al., 2012).

Computer-Aided Drug Design (CADD) covers a broad range of applications spanning the drug discovery pipeline although these are highly clustered in the early phase. The main purpose of using CADD is to speed up and rationalize the drug design process while reducing costs (Taff et al., 2014). Hence, CADD is fast and automatic compared to traditional drug screening.

A pharmacophore model can be established either in a structure-based pharmacophore analysis by probing possible interaction points between the macromolecular target and ligands, or in a ligand-based pharmacophore analysis by superimposed a set of active molecules and extracted the common chemical features that are essential for their

bioactivity (Yang, 2010). The pharmacophore modelling was applied using LigandScout 4.3 software. LigandScout 4.3 software is an emerging and evolving modern computer science that provides an opportunity for researchers to go through a vast volume of data to identify whether a particular compound is suitable for pharmaceutical usage (Lallier, 2014). Pharmacophore modelling helps a lot in the detection of 2D and 3D molecules by identifying several key elements in the components of the sample (Sheridan & Kearsley, 2002). According to Qing et al. (2014), the 3D pharmacophore analysis was able to describe the action of a series of ligands, which is one of the computational chemistry's most important contributions to drug discovery. In addition, the chemical features of the pharmacophore model could be hydrogen-bond donor (HBD), hydrogen-bond acceptor (HBA), hydrophobic (HY), positive ionisable (PI) area, negative ionisable (NI) area and aromatic (Ar) (Salemme, 2001).

## **1.2 Problem Statement**

The existing studies of other parts of *N. fruticans* Wurmb such as leaves, unripe endosperm, ripe endosperm, fruit husk had been mainly focused on antioxidant properties (Aziz & Jack, 2005; Prasad et al., 2013). Nipa palm sugar has been used as an alternative sweetener by local people to make desserts and sweets, and it might cause disease to the human being which is diabetes mellitus. Diabetes mellitus is a metabolic disorder that is caused by a defect in insulin secretion and insulin action (Bastaki, 2005). In this study, nipa palm (*N. fruticans* Wurmb) sugar extracts were analysed to determine its chemical constituents whether it possesses the ability to inhibit alpha-amylase. Other than that, nipa palm sugar compounds also were analysed by using computer aided drug design (CADD)

which are ligand-based pharmacophore model and structure-based pharmacophore model. CADD is a computational method which can discover, improve, learn drugs and any other related biologically active molecules (Zhang et al., 2017). In addition, the main target of CADD is to predict whether a given molecule will bind to a specific target (Yang, 2010). It helps to determine the compound which possesses a potential to be alpha-amylase inhibition. CADD was used due to its higher accuracy of results, faster and inexpensive cost compared to traditional methods. Thus, the research about the antidiabetic properties which is by inhibiting alpha-amylase of nipa palm sugar extracts could increase the medicinal value of the nipa palm tree.

### **1.3 Objectives**

The objectives of this research are:

- i. To determine the moisture content, ash content and pH of the nipa palm sugar extracts.
- ii. To quantify the phytochemical constituents in nipa palm sugar extracts.
- iii. To evaluate the antioxidant, alpha-amylase inhibition and toxicity activities of nipa palm sugar extracts.
- iv. To analyse the antidiabetic properties of nipa palm sugar extracts by using pharmacophore modeling.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 *Nypa fruticans* Wurmb

##### 2.1.1 Plant Profile

*Nypa fruticans* Wurmb (Figure 2.1) belong to the family of Arecaceae and grow abundantly in river estuaries and water environments where salt and freshwater mingle (Tamunaidu & Saka, 2011). Arecaceae consists of 181 genera and 2600 species and majority of them live in subtropical and tropical climates (Christenhusz & Byng, 2016). There are six major subfamilies of the Arecaceae which are Calamoideae, Coryphoideae, Nypoideae, Arecoideae, Phytelephantoideae and Ceroxyloideae (Dransfield, 2015). Hence, *N. fruticans* Wurmb belongs to the Nypoideae subfamily. Some of the significant species in the Arecaceae family are shrubs, tropical climbers and trees commonly known as palm trees.



**Figure 2.1:** *Nypa fruticans* Wurmb's tree

*N. fruticans* Wurmb which also being called as mangrove plants as it grows in a mangrove environment. According to Ebana et al. (2015), the habitat of *N. fruticans* Wurmb is considered as unique as it does not need to compete with food sources and its growth is also sustainable. Table 2.1 shows the taxonomy of *N. fruticans* Wurmb.

**Table 2.1:** The taxonomy of *N. fruticans* Wurmb (CABI, 2006)

Rank	Scientific Name
Domain	Eukaryota
Kingdom	Plantae
Phylum	Spermatophyta
Subphylum	Angiospermae
Class	Monocotyledonae
Order	Arecales
Family	Areaceae
Genus	<i>Nypa</i>
Species	<i>Nypa fruticans</i>

### 2.1.2 Vernacular Names of *Nypa fruticans* Wurmb

*Nypa fruticans* Wurmb had many vernacular names because every country had their own names. Therefore, Table 2.2 shows the vernacular names for *Nypa fruticans* Wurmb.

**Table 2.2:** The vernacular names of *Nypa fruticans* Wurmb (Baja-Lapis et al., 2004)

Countries	Vernacular names
Thailand	chak, at-ta
Vietnam	dua la, dua muoc
Myanmar	dani
Singapore	atap palm
Indonesia	nipah, buyuk, bobo
Malaysia	nipah
Papua New Guinea	biri-biri